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ENVIRONMENTAL QUALITY RESEARCH FISH AND AUFWUCHS BIOASSAY

ANNUAL REPORT

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AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 80-33.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

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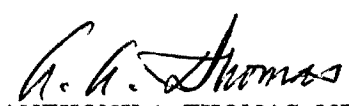

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

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This report contains the results of research efforts of a project concerned with defining the effects of potential environmental contamination resulting from the use of certain Air Force materials on fresh water fish. Materials Being evaluated include JP-4 and JP-8. Techniques for exposing organisms to these substances are discussed and results of such exposures are presented.

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SUMMARY AND CONCLUSIONS

1. This report deals with the toxicity of the fuels JP-4 and JP-8 to the warm water fish, golden shiner (Notemigonus chrysoleneas).

2. Gas chromatographic methods of solvent extracts have been developed for the qualitative and quantitative analysis of both fuels in aqueous systems.

3. The response of the golden shiner was examined in acute static bioassays of water saturated with soluble fuel and water in contact with pools of floating fuel.

4. The effect of water hardness in the range 9-270 mg $CaCO_3$/ℓ on JP-8 toxicity was examined in three bioassays utilizing reconstituted soft water, reconstituted hard water, and Richmond Field Station (RFS) tap water saturated with soluble fuel and renewed each day. These studies supported the following conclusions:

   a. Acute toxicity as assessed by the 96-hr LC50 values was not significantly affected by total hardness:

Water	Total Hardness mg $CaCO_3$/ℓ	LC50 mg/ℓ	95% Confidence Limits, mg/ℓ
Soft	9	8.42	6.83 - 10.39
Tap	28	8.53	7.65 - 9.52
Hard	270	7.73	6.69 - 8.94

   b. The aqueous saturation concentration of soluble JP-8 was 12.1 mg/ℓ ± 1.162 mg/ℓ.

   c. Volatilization losses during the conduct of acute static bioassays reduced the saturated JP-8 concentration from 12.1 to 8.5 mg/ℓ in a 24-hr period.

   d. Volatilization did not preferentially remove a singly toxic component of JP-8 fuel from solution. This was determined by pre-aeration of a saturated JP-8 solution for a 24-hr period prior to fish exposure and renewal of the pre-aerated solution each day. The toxicity was similar to the solutions containing approximately 8.5 mg/ℓ JP-8 which were not pre-aerated.

   e. At sublethal fuel concentrations, fish exhibited stress symptoms characterized by their position in the assay vessel which was a function of fuel concentration. In a series of assay vessels containing successively higher soluble fuel concentrations, the fish positioned themselves at successively shallower depths.

f. Other significant stress symptoms were the appearance of a dark body coloration and a tendency towards vertical orientation rather than the normal horizontal swimming position.

g. Stressed fish readily recovered during a static bioassay as fuel volatilized and even moribund fish recovered when placed in toxicant-free water.

5. Results of studies on the toxicity of water soluble JP-4 components led to the following conclusions:

   a. JP-4 (96-hr LC50, 3.8 mg/ℓ) is about twice as toxic to golden shiners as JP-8 (mean 96-hr LC50, 8.2 mg/ℓ).

   b. The mean volatility loss of soluble JP-4 components in a 24-hr period was 35.4% ± 11.4%.

   c. Stress symptoms were similar to those noted for water soluble JP-8 components.

   d. The saturation concentration of soluble JP-4 was 15.7 mg/ℓ.

6. Results of studies in which water was in contact with pools of floating fuel led to the following conclusions:

   a. The JP-8 96-hr LC50 of 316 µℓ/ℓ (95% confidence limits 163 - 610 µℓ/ℓ) was not significantly different from the JP-4 96-hr LC50 of 570 µℓ/ℓ (95% confidence limits 400 - 800 µℓ/ℓ).

   b. JP-4 was significantly more toxic than JP-8 after 24 and 48 hours of exposure.

   c. JP-4 initially solubilizes more rapidly but at 96 hours in the 500 µℓ/ℓ range (which is crucial in toxicity studies) the soluble JP-8 concentration is much higher (8.0mg/ℓ) than the soluble JP-4 concentration (4.5 mg/ℓ).

   d. Physical encounters of fish with floating fuel was not a significant factor in mortality as previously noticed in the studies of other fuels such as RJ-4 and RJ-5.

   e. Stress symptoms were evident at nonlethal fuel levels, such as swimming at shallower depths, dark body coloration, spasmodic movement and the appearance of a white material in the water probably resulting from extraction of fats from the fish by fuel or the excretion of mucus.

PREFACE

The research reported herein was conducted at the Sanitary Engineering Research Laboratory, University of California at Berkeley, under the terms of contract F33615-76-C-5005 with the Air Force. The contract monitor was Lt Col Roger C. Inman, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. Professors David Jenkins and Robert C. Cooper were the Principal Investigators. Mr. Stephen Klein was the project manager. Ms. P. C. Ulrichs was responsible for conduct of bioassays. Mr. Jonathan Palm, Ms. Nancy Quan and Mr. Steven Krugel, candidates for the M.S. degree, were research assistants.

TABLE OF CONTENTS

SUMMARY AND CONCLUSIONS 1

PREFACE 3

LIST OF TABLES 7

LIST OF FIGURES 8

I. INTRODUCTION 9

II. MATERIALS AND METHODS . . . . .

Fuels 9

Water Soluble Fuel 10

Intact Fuel 10

Static Bioassays 10

LC50 Determination 11

Golden Shiners 11

Expression of Fuel Concentrations 11

Gas Chromatographic (GC) Analysis of Fuels 11

Temperature Program Operation 11

Isothermal Operation 12

Standard Curves 12

Chromatograms 12

Internal Standard 12

Extraction of Fuel from Water 19

Reconstituted Soft Water 19

TABLE OF CONTENTS (continued)

		Page
	Reconstituted Hard Water	19
	Tap Water	19
III.	JP-8 TOXICITY	
	Introduction	20
	Results of Soluble JP-8 Bioassays	20
	Soft Water	20
	Tap Water	21
	Hard Water	22
	Discussion	23
	Results of Pure Fuel Bioassays	27
	Discussion	
IV.	JP-4 TOXICITY	
	Introduction	30
	Results of Soluble JP-4 Bioassays	30
	Comparative Toxicity of Water Soluble JP-4 and JP-8	33
	Results of Pure Fuel Bioassays	33
	Discussion	33
V.	REFERENCES	38

LIST OF TABLES

Table	Title	Page
1.	Toxicity of Water Soluble JP-8 Components to Golden Shiners in Reconstituted Soft Water	21
2.	Acute Toxicity of Soluble JP-8 to Golden Shiners in Richmond Field Station Tap Water	22
3.	Acute Toxicity of Soluble JP-8 to Golden Shiners in Reconstituted Very Hard Water	26
4.	Statistical Analysis of Effect of Water Hardness on Toxicity of Soluble JP-8 to Golden Shiners	26
5.	Survival of Golden Shiners in Static Bioassay with Floating Pools of Pure JP-8 Fuel in RFS Tap Water	29
6.	Survival of Golden Shiners Exposed to Soluble JP-4 . . .	32
7.	Volatility of JP-4 During 24-hour Period	32
8.	Comparison of Acute Toxicity of Water Soluble Components of JP-8 and JP-4 to Golden Shiners	35
9.	Comparison of Acute Toxicity of Pure JP-8 and JP-4 to Gold Shiners	35

LIST OF FIGURES

Figure	Title	Page
1.	Standard Curve for JP-8	13
2.	Standard Curve for JP-4	14
3.	Chromatogram of JP-8 Using Temperature Programming . .	15
4.	Chromatogram of JP-8 Using Isothermal Operation	16
5.	Chromatogram of JP-4 Using Temperature Programming . .	17
6.	Chromatogram of JP-4 Using Isothermal Operation	18
7.	Fish Survival in 24-hour Pre-Aerated 100% Soluble JP-8 (Soluble JP-8 Concentration of 8.5 mg/ℓ)	24
8.	Effect of Water Hardness on Toxicity of Soluble JP-8 to Golden Shiners	25
9.	Dissolution of JP-8 at a Series of Volumetric Additions	28
10.	Acute Toxicity of Soluble JP-4 Components to Golden Shiners (24 Hour Solution Renewal	31
11.	Comparison of Soluble JP-4 Acute Toxicity Studies (Test Organism: Golden Shiners)	34
12.	Solubilization of a Series of Volumetric Additions of Pure Fuel JP-4 with Time	36

I. INTRODUCTION

Studies included in this report are directed toward providing information on the toxicity of certain Air Force materials to aquatic life. Of the two jet fuels, JP-4 and JP-8, currently under investigation, most of the work completed to date has been on JP-8.

The experimental protocol established for defining the environmental effects of a specific material includes both continuous-flow chronic and static bioassays on cold water fish species, warm water fish species and Aufwuchs. The studies to date on JP-4 and JP-8 are still in progress. Results are reported on a series of acute toxicity studies with the warm water fish, golden shiner (Notemigonus chrysoleneas). The first group of experiments on the effect of water hardness on the acute toxicity of JP-8 and a second group of studies examines the effect of various modes of fuel exposure.

There are several forms in which fuel may be present in the aquatic environment. A fuel spill may result in the formation of a floating pool of pure fuel. An emulsion of fuel droplets and fuel components in true solution may also exist. Acute bioassays on pools of fuel and the soluble fraction of fuel have been completed for JP-8.

Some static bioassays on golden shiners using JP-4 were reported in the 1976 Annual Report, using the three aforementioned modes of fuel exposure. Since then the procedure for saturation of water with soluble fuel components has been revised. The revised procedure has been applied to JP-4 to provide valid comparison with JP-8 results obtained using the revised water saturation method.

As a necessary corollary to the toxicity studies, development work has been conducted on gas chromatographic (GC) procedures for the qualitative and quantitative analysis of fuels.

Results obtained from the experiments on saturation of water with soluble JP-4 in which the JP-4 was determined quantitatively by GC have been applied to the previous work with JP-4 which was reported in volumetric concentrations because an analytical test was not previously available.

II. MATERIALS AND METHODS

Fuels

The jet fuels JP-4 and JP-8 were supplied by the Aerospace Medical Research Laboratory, USAF.

JP-4 is a kerosene-type aviation fuel consisting of a complex mixture of aliphatic and aromatic hydrocarbons. The batch supplied contained weight percentages of 0.3% benzene, 0.2% toluene and 0.8% mixed xylenes and has a density of 0.746 g/mℓ.

JP-8 is a kerosene-type aviation fuel consisting of a complex mixture of aliphatic and aromatic hydrocarbons with a predominance of denser components. Its specified density is in the range 0.775 to 0.840 g/mℓ.

Both fuels may contain various additives such as antioxidants, metal deactivators, corrosion inhibitors and icing inhibitors.

Water-Soluble Components of a Fuel

The water soluble components of a fuel were prepared by saturating 18 ℓ of a 5% fuel in water mixture over a period of 24 hours in narrow-mouth 5- gal carboys. The air space volume was 2 ℓ and the air-to-liquid volume ratio was 1:9. Each carboy was plugged with a rubber stopper through which a glass sampling tube was placed. The carboys were mixed continuously for 24 hours with magnetic stirrers at a rate which did not create a vortex on the surface and avoided physical dispersion of fuel droplets in the water. Tests indicated that complete saturation could be achieved at this fuel-to-water volumetric ratio and exposure time. After 24 hours the aqueous fuel solution was removed through the sampling devices from a point near the bottom of the carboy. The first several hundred ml were discarded. The water was replaced and saturated with the same pool of fuel to meet the fuel solution requirements for the following day. The type of water used depended on the aim of the particular experiment, and was either reconstituted soft water, reconstituted hard water, or Richmond Field Station tap water.

Intact Fuel

Intact fuel was added to static assay vessels and permitted to float on the water surface. In these assays the fish were introduced into the assay vessels before the fuel was added so that direct contact between fish and floating fuel was avoided.

Static Bioassays

Static bioassays were conducted in 20-ℓ capacity wide-mouth glass jugs filled to the 15-ℓ mark and provided with the minimum amount of aeration with filtered air to maintain the dissolved oxygen concentration above 4 mg/ℓ throughout the bioassay. The fish weight/dilution water ratio was a minimum of 1 ℓ per g of fish as specified in Standard Methods (1970). Fuel volatility loss was ameliorated by daily solution renewals and by covering assay vessels with aluminum foil.

LC50 Determination

LC50 values were determined by the Standard Methods (1970) technique and by the Reed-Muench method (Woolf, 1968) when 95% confidence limits were computed.

Golden Shiners

Golden shiners (Notemigonus chrysoleneas) were obtained from the Sierra Bait Company, a commercial fish hatchery. They were acclimated to reconstituted water (either hard or soft) for a minimum of 6 weeks before use in bioassays. During acclimation, 70% of the water in the holding tank was replaced twice each week.

The fish used in the soft water and tap water bioassays had an average length of 4.19 ± 0.08 cm (standard error of the mean) and a mean wet weight of 1.15 g. Fish used in the hard water bioassays had an average length of 4.55 ± 0.08 cm and a mean wet weight of 1.27 g.

Expression of Fuel Concentrations

The fuel concentration for a water saturated with fuel was expressed as 100% (saturated) or some dilution of 100%. The fuel concentrations in mg/ℓ were measured by gas chromatography for the 100% solution so that the concentration for any dilution of the 100% solution the mg/ℓ could be computed. In work with pure fuel the volumetric additions are reported as µℓ of fuel per ℓ of dilution water. The concentration of fuel that dissolves in aqueous bulk solution from a surface pool of fuel was determined by GC and reported as mg/ℓ .

Gas Chromatographic (GC) Analysis of Fuels

Aqueous fuel concentrations were determined by GC analysis using a Fisher Model 4800 gas chromatograph with dual flame ionization detectors and 20 ft x 1/8 in o.d. stainless steel columns of 10% SE30 on 80/100 Chrom W. Accessory GC equipment included a Fisher Series 5000 Recordall recorder and an Autolab minigrator for digital integration of peaks.

For determination of changes in the fuel composition, it was necessary to use temperature programming, but for reliable quantitation of total fuel concentration, isothermal operation was better. Temperature changes apparently produced electrical noise, resulting in erratic results during programming. Two approaches were examined for their suitability in quanitfying the fuels: The first considers only four major peaks while the second considers the total area of all peaks.

Temperature Program Operation. For JP-8 the most suitable program for peak differentiation was: 60°C for 10 min; increase to 180°C at 4°C/min; final delay at 180°C for 20 min. The JP-4 program differed only in having an initial delay at 60°C of 18 min.

Isothermal Operation. A temperature of 160° C was used for quantitative analysis of both JP-8 and JP-4. For both isothermal and temperature-programmed operation, the injection and detector temperatures were 270° C and the N_2 carrier gas flow rotameter setting was 3.0 at 80 psig. The detector flame H_2 flow was set at a rotameter reading of 4.5.

Standard Curves for the GC analysis of JP-8 and JP-4 are linear in the range of 10^{-2} to 10^{-4} g for both the total peak area and the selected major peaks of JP-8 (Figure 1) and the total peak area of JP-4(Figure 2).

Chromatograms of JP-4 and JP-8 (Figures 3 - 6) show that temperature programming provides peak separation and is necessary for evaluating qualitative changes in the fuel composition. Under isothermal operation at 160°C, peaks in the C_6-C_{10} range elute rapidly and immediately after the solvent peaks.

Internal Standard. The internal standard selected for JP-8 was n-pentadecane, C_{15}, which elutes after the JP-8 components. The relationship between JP-8 mass and mass of C_{15} is:

$$M_{JP-8} = M_{C_{15}} \cdot \frac{A_{JP-8}}{A_{C_{15}}} \cdot K$$

where M_{JP-8} = mass of JP-8

$M_{C_{15}}$ = mass of C_{15}

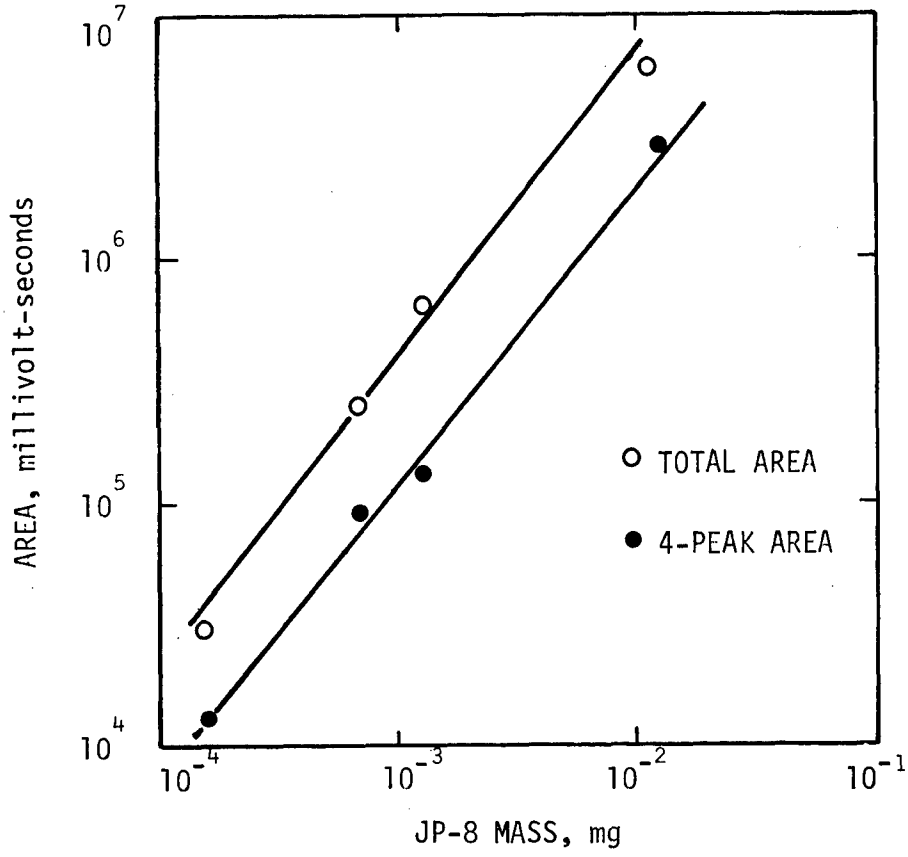
A_{JP-8} = area of JP-8

$A_{C_{15}}$ = area of C_{15}

K = constant .

The constant, K, was determined by injecting a series of known masses of JP-8 and C_{15} and measuring their respective peak areas using isothermal operation, The results of this calibration for a series of five mixed samples was a mean K = 1.17,with a coefficient of variation of 4.8%. Using temperature programming the mean K value was 1.06 with a coefficient of variation of 14%. From these data it was concluded that the isothermal technique was the more reproducible technique.

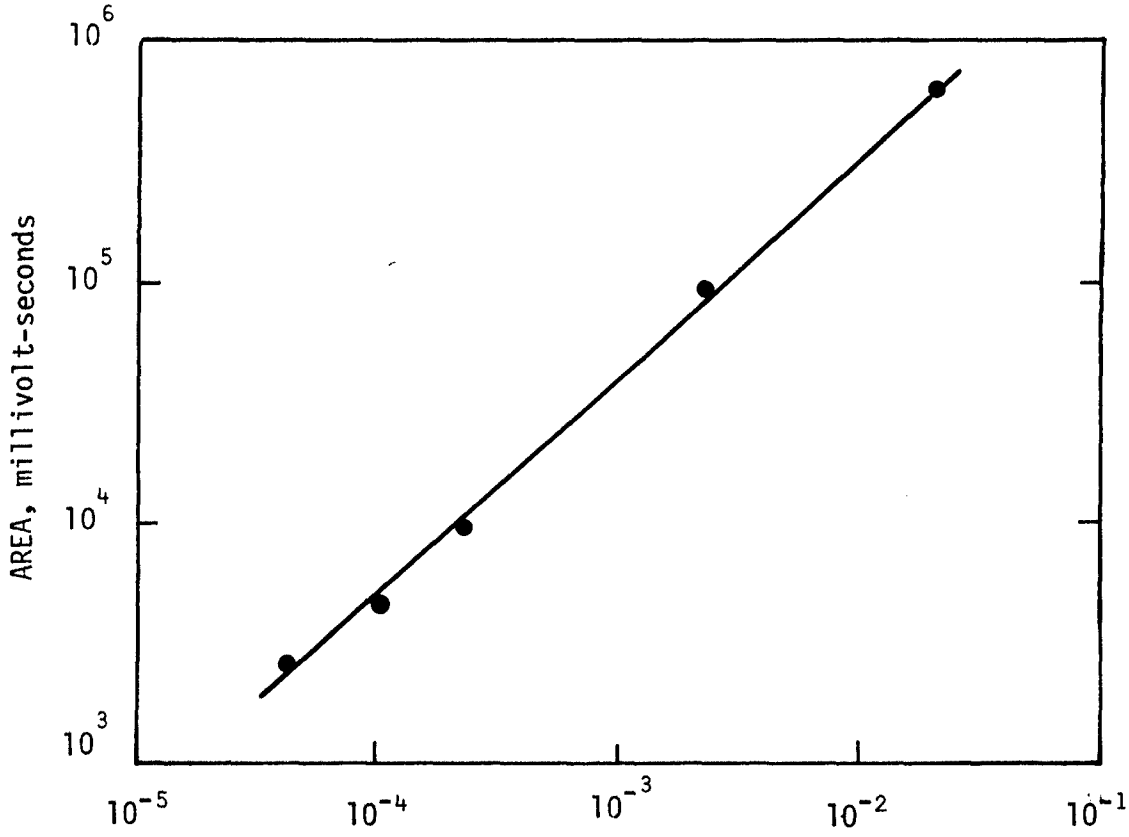
The internal standard selected for JP-4 was n-tetradecane(C_{14}). Although this compound is present in JP-4 fuel, it does not dissolve to a detectable level in aqueous solution. Determining the K value for JP-4 and C_{14} involved a correction step of subtracting the mass of C_{14} present in the pentane solutions of JP-4. This was accomplished by analyzing



GC Conditions

Dual Column Operation
Temperature Programming
Injector Temperature 270° C
Detector Temperature 270° C
Injection Volume 1.0 μℓ

FIGURE 1. STANDARD CURVES FOR JP-8



GC Conditions

Dual Column Operation
Temperature Programming
Injector Temperature 270° C
Detector Temperature 270° C
Injection Volume 1.0 µℓ

FIGURE 2. STANDARD CURVE FOR JP-4

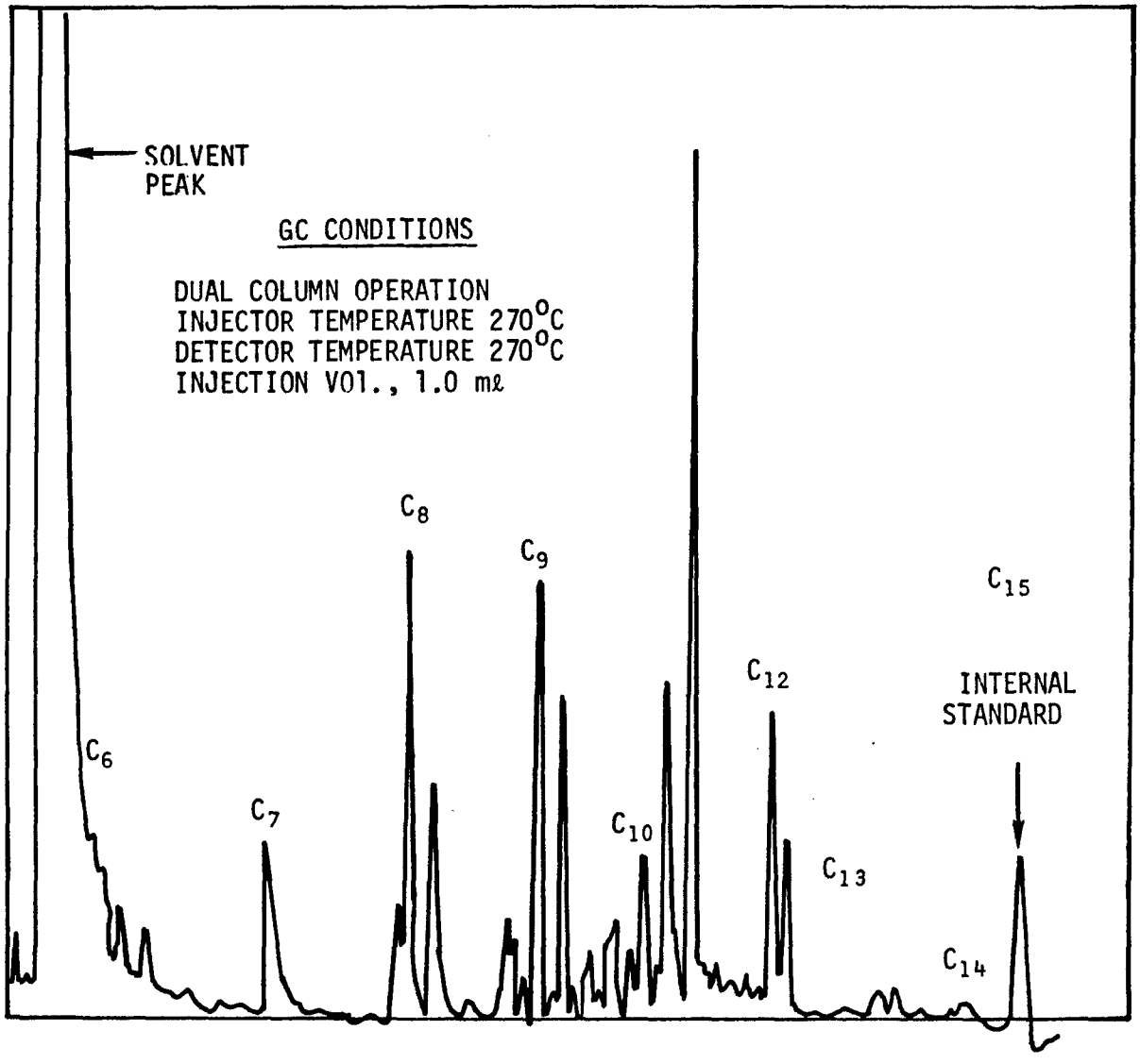


FIGURE 3. CHROMATOGRAM OF JP-8 USING TEMPERATURE PROGRAMMING

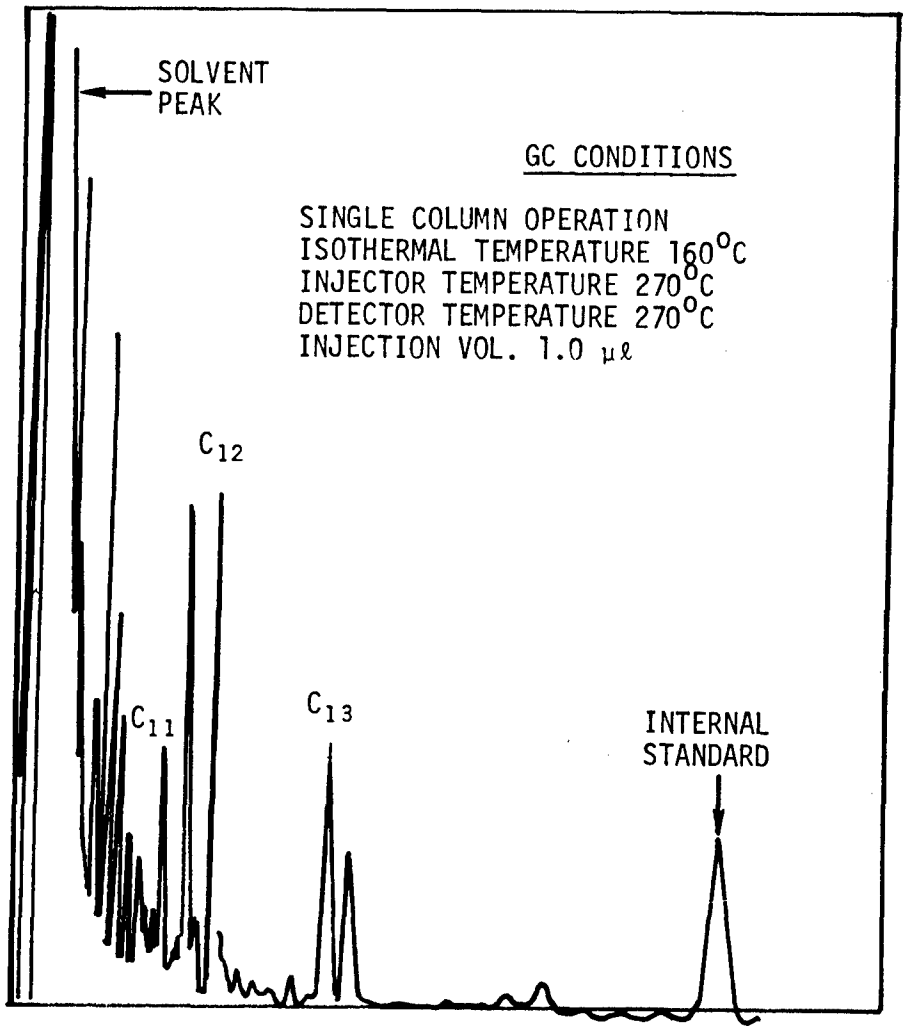


FIGURE 4. CHROMATOGRAM OF JP-8 USING ISOTHERMAL OPERATION

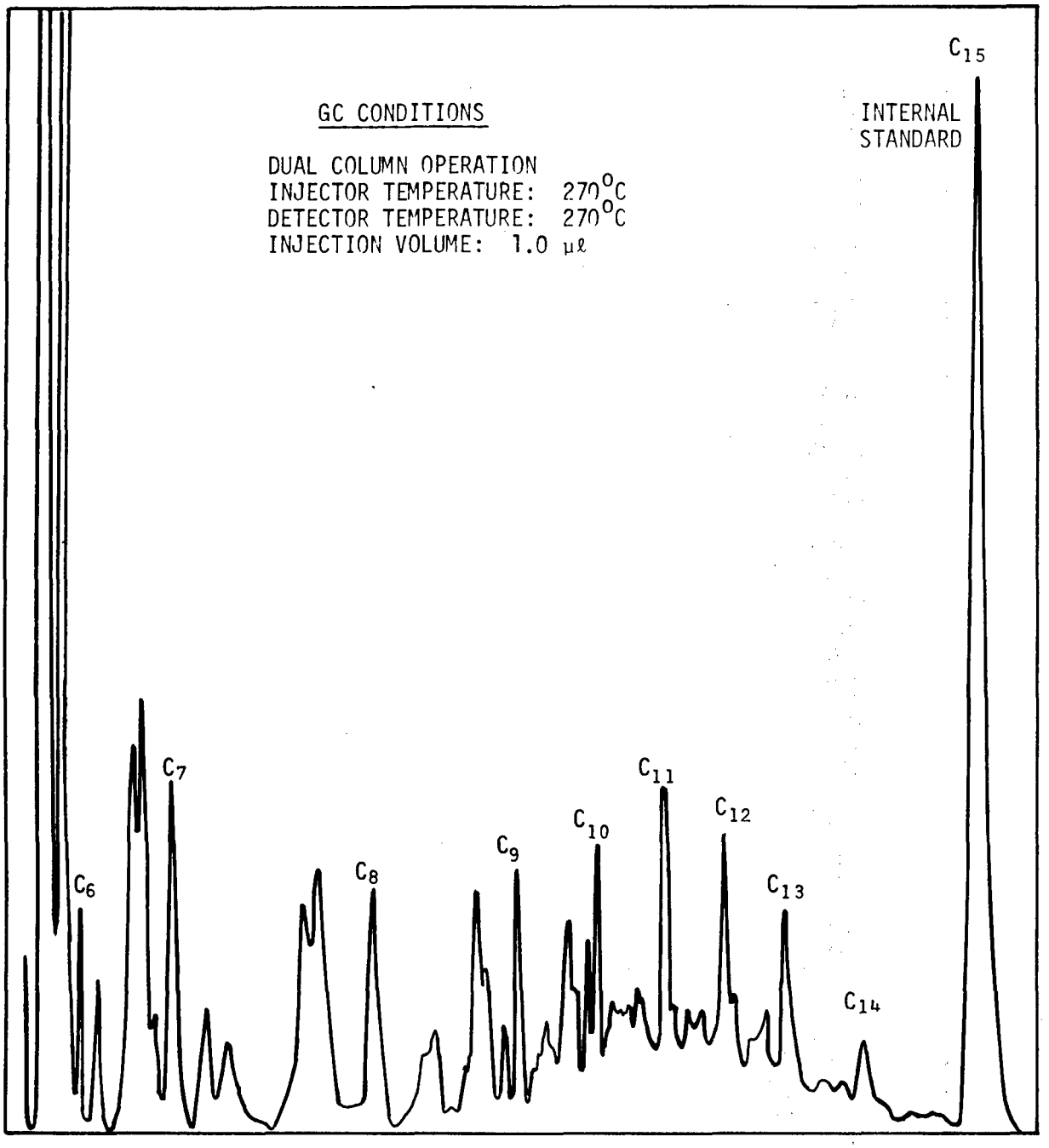


FIGURE 5. CHROMATOGRAM OF JP-4 USING TEMPERATURE PROGRAMMING

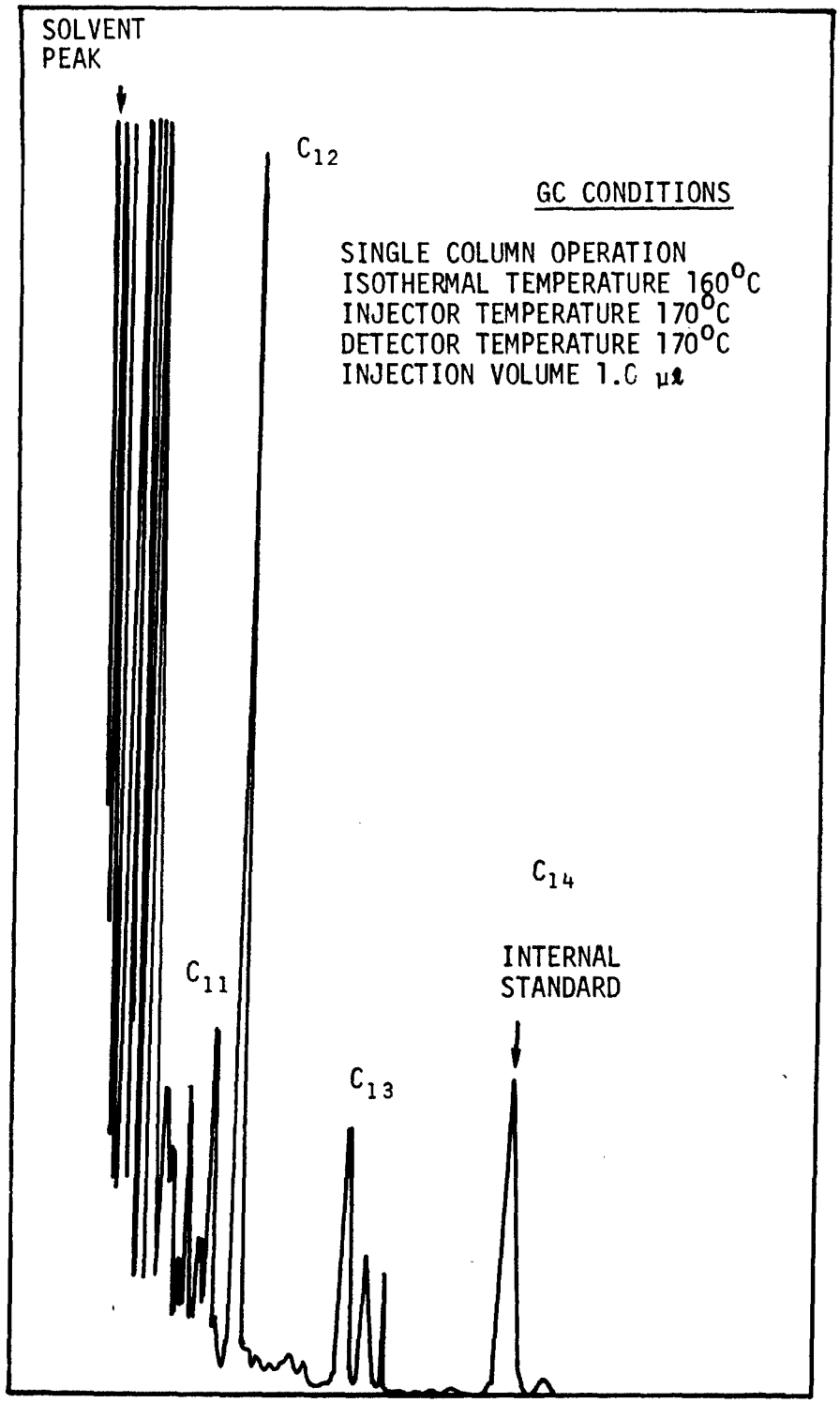


FIGURE 6. CHROMATOGRAM OF JP-4 USING ISOTHERMAL OPERATION

pentane solutions of JP-4 before and after internal standard addition. The K value determined in this fashion was 1.1146 (standard deviation 0.071, coefficient of variation 6.37%).

Extraction of Fuel from Water

The following method for extracting fuel from water was developed:

a. To 300 mℓ sample, add 25 mℓ nanograde n-pentane and shake 2 min by movement of the separatory funnel through a 90° arc at a rate of one complete downward and upward movement per sec.

b. If necessary to avoid emulsions, add 50 mℓ salt solution (100 g NaCl/ℓ distilled water) to original sample.

c. Allow to separate for approximately 10 min, remove aqueous layer, and dewater the pentane layer by passing it through a 3-cm diameter by 3.5-cm deep column of anhydrous sodium sulfate.

d. Repeat steps a and c and combine extracts.

e. Concentrate the combined extract on a rotary evaporator to either 10 mℓ or 1 mℓ (concentration factor of 30-fold or 300-fold, respectively).

f. Perform GC analysis.

Reconstituted Soft Water

Very soft reconstituted water was formulated in accordance with EPA Bulletin 660/3-75-009 (1975) which stipulated the following compound concentrations added to distilled water: $NaHCO_3$, 12.0 mg/ℓ; $CaSO_4 \cdot 2H_2O$, 7.5 mg/ℓ; $MgSO_4$, 7.5 mg/ℓ; KCl, 0.5 mg/ℓ. This water was stated to have a hardness of 10-13 mg as $CaCO_3$/ℓ. All chemicals were reagent grade except for $NaHCO_3$ which was U.S.P.

Reconstituted Hard Water

From the source cited above, hard water was reconstituted by adding the following concentrations of compounds to distilled water: $NaHCO_3$, 384 mg/ℓ; $CaSO_4 \cdot 2H_2O$, 240 mg/ℓ; $MgSO_4$, 240 mg/ℓ; KCl, 16 mg/ℓ. This water was stated to have a hardness of 280-320 mg as $CaCO_3$/ℓ.

Tap Water

For bioassays utilizing Richmond Field Station tap water, the water was dechlorinated by passage through a carbon filter or by vigorous aeration for 24 hours with filtered air.

III. JP-8 TOXICITY

Introduction

Static bioassays were conducted on golden shiners using two modes of fuel exposure: contact with water soluble fractions of JP-8 and exposure to floating pools of pure fuels. In the first type of exposure the effect of water hardness on toxicity was investigated by utilizing waters with total hardness ranging from 9-270 mg as $CaCO_3/\ell$.

Fuel volatility loss during the 96-hr test periods was ameliorated by renewing the solutions daily.

Results of Soluble JP-8 Bioassays

Soft Water. Reconstituted soft water (total hardness, 9 mg $CaCO_3/\ell$) was saturated with JP-8 and the saturated water used to prepare percent dilutions of 100, 79, 50, 30, 20, 12 and 0. There were two assay vessels containing the 50% dilution, one of which was renewed with fresh solution daily while the other was not renewed throughout the assay period. All of the other dilutions were renewed daily.

Based on 5 GC measurements of the water saturation concentration of JP-8, the JP-8 concentration in the 100% solution was assumed to be 12.1 ± 1.162 mg/ℓ. The JP-8 concentration in the serial dilutions was computed from this value and the dilutions.

There was no mortality at the 50% dilution of water soluble JP-8 (concentration estimated to be 6.1 mg JP-8/ℓ)(Table 1). The 96-hr LC50 was 70% of saturation or an estimated water soluble JP-8 concentration of 8.4 mg/ℓ.

This result must be interpreted with caution, because there was visual evidence of stressed fish behavior in all the assay vessels containing JP-8 solution. The fish showed evidence of stress when initially exposed to the solution, but as time progressed throughout each 24-hr exposure period, the symptoms became less pronounced. Presumably this was due to the progressive loss of JP-8 from solution by volatilization. The stress symptoms included a general dark body coloration, swimming near the surface, and in the pronounced state, the fish were at an angle of between 45° and 90° to the water surface. In the very pronounced stage, the fish appeared to be moribund on the bottom of the vessel. The fish in the renewed 50% dilution showed all of these stages progressively through a 96-hr assay, from mild stress symptoms for the first 48 hours to pronounced symptoms by 72 hours. Five of the fish were moribund after 96 hours. Three of these five had a red coloration and the remaining five were swimming at an angle of 45° to the water surface. All of these fish recovered subsequently when held in toxicant-free water.

Table 1

Toxicity of Water Soluble JP-8 Components to Golden Shiners in Reconstituted Soft Water

JP-8 Conc.		Fish Survival, no.				
Volume %	mg/ℓ	Time, hr.				
		0	24	48	72	96
0	0	10	10	10	10	10
12	1.5	10	10	10	10	10
20	2.4	10	10	10	10	10
32	3.9	10	10	10	10	10
50	6.1	10	10	10	10	10
79	9.6	10	6	6	4	3
100	12.1	10	0	0	0	0
50*	6.1	10	9	9	9	9

* solution not renewed

The location of the golden shiners in the assay vessels was directly related to the fuel concentration. The control fish remained at a depth of 16-22 cm beneath the surface throughout the 96-hr period while fish in assay vessels containing fuel solution swam at shallower depths. At successively higher fuel concentrations, the fish were positioned at successively higher locations in the assay vessels. As the time of exposure to the fuel progressed throughout the assay period, the fish in all vessels moved upwards until they were at the surface by the end of 96 hours (with the exception of the fish exposed to the lowest fuel concentration of 12% of saturation which had moved up to a depth of 8-12 cm from the surface by 96 hours). This phenomenon is referred to in subsequent studies as stratification.

Tap Water. The purpose of this study was to compare the toxicity of water soluble JP-8 to golden shiners in Richmond Field Station (RFS) tap water to that in soft reconstituted water. RFS water had a total hardness of 28 mg $CaCO_3$/ℓ.

Assays were conducted at volumetric dilutions of water saturated with the soluble components of JP-8 of 100, 79, 63, 50, 40, and 0%. Two assays were conducted with 100% water soluble JP-8; one utilized the 24-hr renewal method while the other retained the same solution for the entire 96-hr period of the bioassay. In all other vessels the 24-hr re-

newal method was used. GC analyses of JP-8 were performed on the unrenewed 100% JP-8 solution at 24, 48, and 72 hours to determine the volatility loss over this period. The solution pH varied between 7.3 and 7.6 and the dissolved oxygen minimum was 4.9 mg/ℓ.

The 96-hr LC50 was on a volumetric basis of 70% or an estimated JP-8 concentration of 8.47 mg/ℓ JP-8(Table 2). This value is almost identical to the 96-hr LC50 obtained in very soft reconstituted water. The 48-hr LC50 was 72% (estimated JP-8 concentration,8.7 mg/ℓ) and the 24-hr LC50 was 83% (estimated JP-8 concentration,10.0 mg/ℓ).

Table 2

Acute Toxicity of Water Soluble JP-8 to
Golden Shiners in Richmond Field Station Tap Water
(Total Hardness, 28 mg $CaCO_3$/ℓ)

Soluble JP-8 Conc.		Fish Survival, no.			
Volume %	mg/ℓ	Time, hr.			
		24	48	72	96
0	0	10	10	10	10
40	4.8	10	10	10	10
50	6.1	10	10	10	10
63	7.6	10	10	8	8
79	9.6	6	2	1	1
100	12.1	1	1	1	1

Fish behavior was similar to that in soft water. In all assay vessels containing JP-8, the fish swam at the surface after 24 hours and remained there for the remainder of the 96-hr assay period.

The concentrations of JP-8 reported in Table 2 are based on five replicated analyses of water saturated with soluble JP-8. The reported concentrations for each dilution were computed from the 100% value. The loss of JP-8 after 24 hours is highly significant. For the renewed solutions,this is representative of the volatility loss experienced each day. The loss was 21.0% after 24 hours, 74% after 48 hours, and 97% by the end of 72 hours.

Hard Water. With the exception of the water hardness and a difference in the size of fish, all other assay conditions were identical to those of the preceding studies with reconstituted soft water and tap water. The statistic at the 99.75% level indicated that the mean fish size was slightly, though significantly, larger (9.4% on a weight basis) in the hard water study (4.55 cm length and 1.27 g wet weight compared to

4.19 cm length and 1.15 g wet weight).

Dilutions of water saturated with JP-8 of 0, 40, 50, 63, 79 and 100% were assayed in reconstituted hard water (total hardness, 270 mg $CaCO_3/\ell$). Because one aspect of this study was to assess the effect of volatilization on the toxicity of JP-8, an additional bioassay vessel was set up containing 100% water soluble JP-8 prepared by 24 hours of pre-aeration . The solution was renewed daily and in every other respect the procedure was identical to the normal bioassay. Thus, if toxic components were readily and preferentially volatilized, there should have been a reduction in toxicity in this vessel.

The 96-hr LC50 was between 50 and 63% of the soluble JP-8 (estimated to be 6.1 to 7.6 mg/ℓ) (Table 3). The computed LC50 for 24 hours was 90% (10.9 mg/ℓ), for 48 hours, 79% (9.6 mg/ℓ) and for 96 hours, 66% (8.0 mg/ℓ) (Standard Methods, 1970). The Reed-Muench method (Woolf, 1968) gave LC50 values of 10.6 mg/ℓ, 9.8 mg/ℓ and 7.7 mg/ℓ, respectively for the above exposure times.

The jar containing 100% water soluble JP-8 components prepared from pre-aerated JP-8 solution was analyzed for JP-8 concentration on two occasions and showed an average of 8.5 mg/ℓ. Thus the 24-hr JP-8 pre-aeration had the effect of reducing the JP-8 concentration by 28% (from 12.1 to 8.5 mg/ℓ). There was no indication from the chromatogram or from the fish survival data that toxic components had been preferentially removed. The toxicity appeared to be similar to that of the dilution prepared with fuel solutions that were not pre-aerated (i.e., between 63% and 79% of saturation). As indicated in Figure 7, the pre-aerated 100% JP-8 required 108 hours to achieve an LC50. The difference in toxicity between the pre-aerated solution and the dilution series is quite small and likely due to experimental or analytical variation. The evidence suggests that the volatilization of JP-8 reduced the overall concentration which in turn lowered the toxicity. Thus there would not appear to be a single toxic component that is preferentially volatilized, or if a toxic component is removed, its effect is too small for resolution by the analytical technique.

Discussion

In soft water (total hardness, 9.0 mg $CaCO_3/\ell$) and in RFS tap water (total hardness, 28 mg $CaCO_3/\ell$), the 96-hours LC50 of water soluble JP-8 was almost identical (8.42 and 8.53 mg/ℓ, respectively). In hard water (total hardness, 270 mg $CaCO_3/\ell$), the 96-hr LC50 was 8.0 mg/ℓ . Statistical analysis of the data by the Reed-Muench method (Table 4 and Figure 8) demonstrates that at the 95% confidence level there is no signficant difference in these values, and it can be concluded that hardness in the range studied has no effect on soluble JP-8 toxicity. Furthermore, the identical JP-8 concentrations in all three of the fully saturated waters (12.1 mg/ℓ) demonstrated the lack of effect of hardness on the solubility of the fuel.

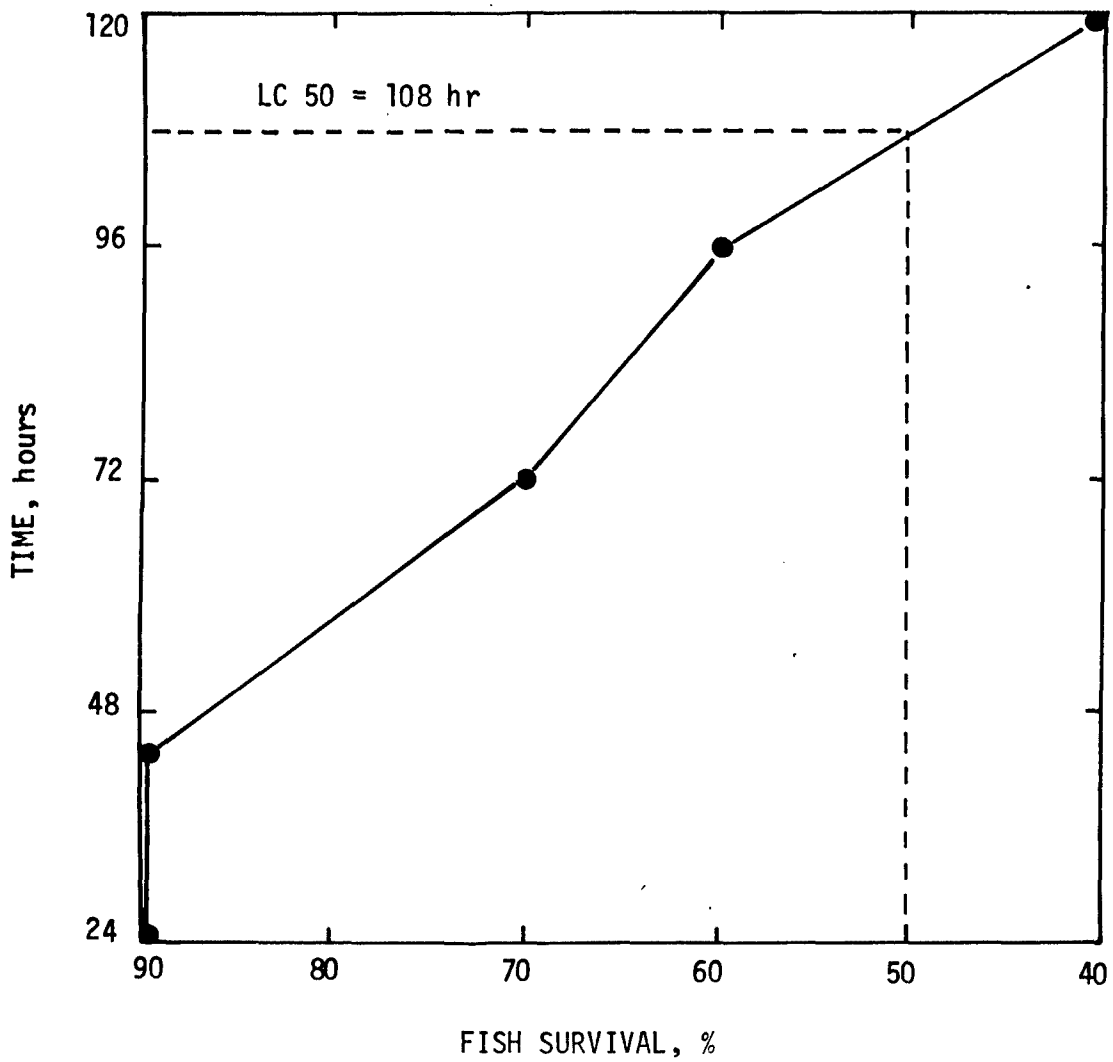


FIGURE 7. FISH SURVIVAL IN 24-hr PRE-AERATED 100% SOLUBLE JP-8 (SOLUBLE JP-8 CONCENTRATION OF 8.5 mg/ℓ)

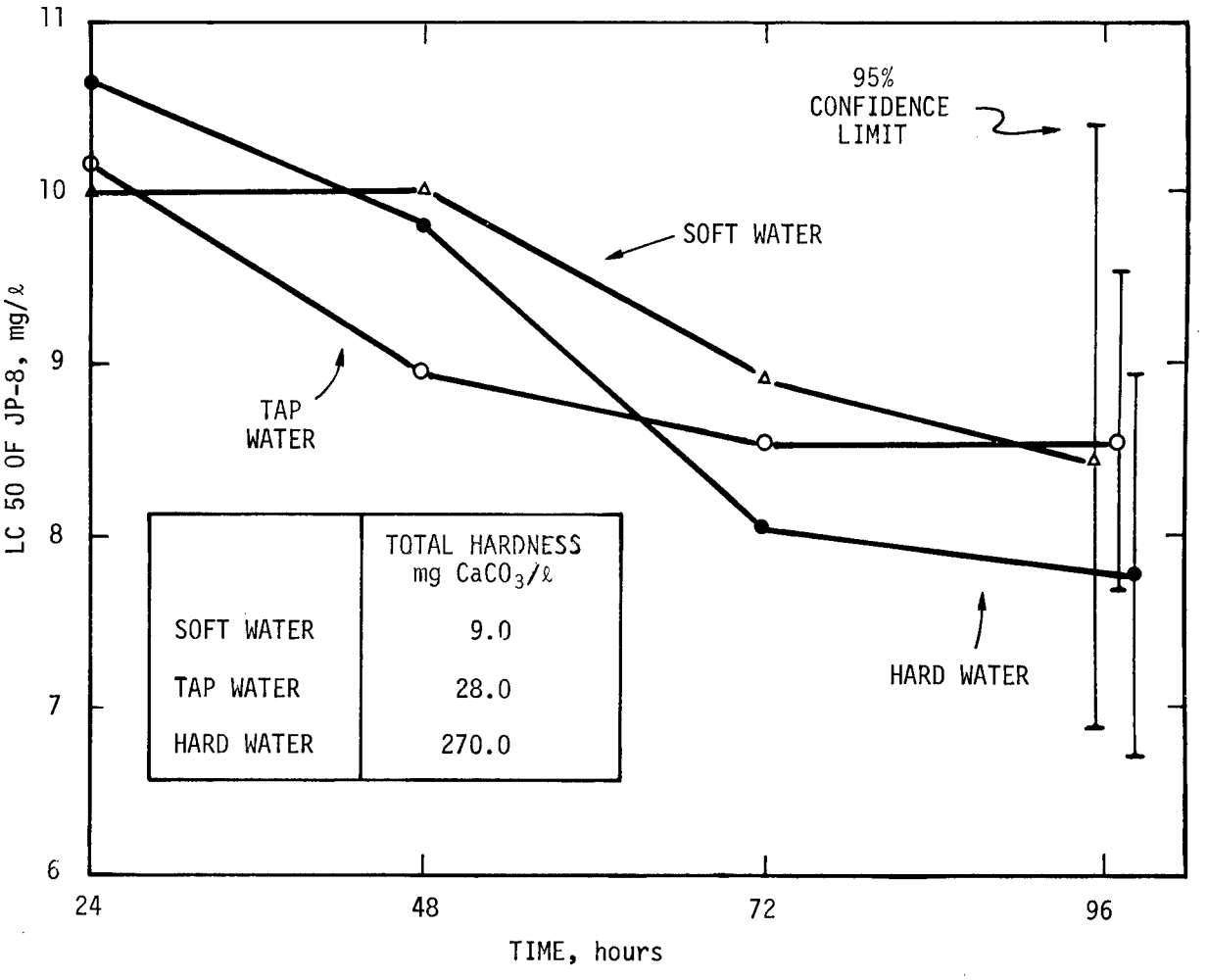


FIGURE 8. EFFECT OF WATER HARDNESS ON TOXICITY OF SOLUBLE JP-8 TO GOLDEN SHINERS

Table 3

Acute Toxicity of Soluble JP-8 to
Golden Shiners in Reconstituted Very Hard Water

JP-8 Conc.		Fish Survival, no.				
Volume %	mg/ℓ	Time, hr.				
		24	48	72	96	120
0	0	10	10	10	10	
40	4.8	10	10	10	10	
50	6.1	9	9	7	7	
63	7.6	10	10	8	6	
79	9.6	7	5	0	0	
100	12.1	3	2	1	0	
100*	8.5	9	9	7	6	4

* Solution aerated 24 hours before utilizing

Table 4

Statistical Analysis of Effect of Water Hardness
on Toxicity of Soluble JP-8 to Golden Shiners

Water	Time hr	LC50 mg/ℓ	95% Confidence Limits, mg/ℓ
Soft	24	9.99	8.18 - 12.21
	48	9.99	8.18 - 12.21
	72	8.90	7.17 - 11.06
	96	8.42	6.83 - 10.39
Tap	24	10.16	8.85 - 11.54
	48	8.94	7.99 - 9.99
	72	8.53	7.65 - 9.52
	96	8.53	7.65 - 9.52
Hard	24	10.61	9.30 - 12.12
	48	9.82	8.53 - 11.31
	72	8.05	7.00 - 9.31
	96	7.73	6.69 - 8.94

Before arriving at a firm value for acute toxicity it will be necessary to conduct continuous-flow acute studies in which volatility losses are less. Experiments are currently planned on JP-8 and JP-4 using a continuous-flow system with a 2-hr residence time in the assay vessel.

Results of Pure Fuel Bioassays

A preliminary range-finding study was performed using 1-gal flasks containing 3 ℓ tap water and fuel concentrations of 0, 10, 50, 100, 500, 1000 and 5000 μℓ JP-8/ℓ dilution water. Each vessel contained three golden shiners and was aerated at the minimum rate to provide sufficient dissolved oxygen over a 120-hr assay period. No deaths occurred at concentrations of ≤ 500μℓ/ℓ; there were no survivors after 96 hours at 1000 μℓ/ℓ.

Based on the results of this preliminary study, a more extensive static bioassay was conducted at the same volumetric concentrations of JP-8 in RFS tap water.

Results of this eight-day assay (Table 5) show that mortality commenced after 48 hours at volumetric JP-8 concentrations of ≥ 500 μℓ/ℓ. By the fourth day there were no survivors at concentrations of 1000 and 5000 μℓ/ℓ. At 500 μℓ/ℓ there was 30% survival.

In all assay vessels containing fuel, there was some indication of stress stratification which increased in severity both as fuel concentration increased and as the time of exposure lengthened. As in the studies on water soluble JP-8 components the orientation of the fish with respect to the surface was a function of fuel concentration, as JP-8 concentration increased the fish swam nearer the surface. Stratification was observed during the first day of exposure and by the end of 48 hours, every fish in all of the assay vessels containing JP-8 were at the surface. Other stress symptoms such as dark coloration, spasmodic movement, were apparent. On the third day, a white material appeared in the water probably from fats and oils extracted from the fish by the fuel or from a mucus discharge. These symptoms were noted even at the lowest JP-8 concentrations. The 96-hr LC50 was 316μℓ/ℓ with 95% confidence limits of 163 μℓ/ℓ to 610 μℓ/ℓ.

These volumetric concentrations can be associated with soluble JP-8 concentrations in mg/ℓ based on GC analyses. Direct analyses could not be made from the assay vessels because the white material caused severe emulsions which interfered with the analysis. Therefore, four vessels containing no fish with concentrations of 100, 500, 1000 and 5000 μℓ/ℓ pure JP-8 fuel were set up and analyzed to determine water soluble JP-8 levels over a 96-hr period. The results (Figure 9) show that a volumetric concentration of 100 μℓ/ℓ JP-8 produced a maximum concentration of 4 mg/ℓ after 96 hours. This explains why there was no mortality in this assay vessel during this period because 4 mg/ℓ JP-8 is within the range of JP-8 concentrations where 100% fish survival was obtained during 96 hours in previous experiments. At 500 μℓ/ℓ and 1000 μℓ/ℓ the concentration of soluble JP-8 was greater than 7 mg/ℓ by 72 hours. This is the time when fish mortality became severe and it is also a JP-8

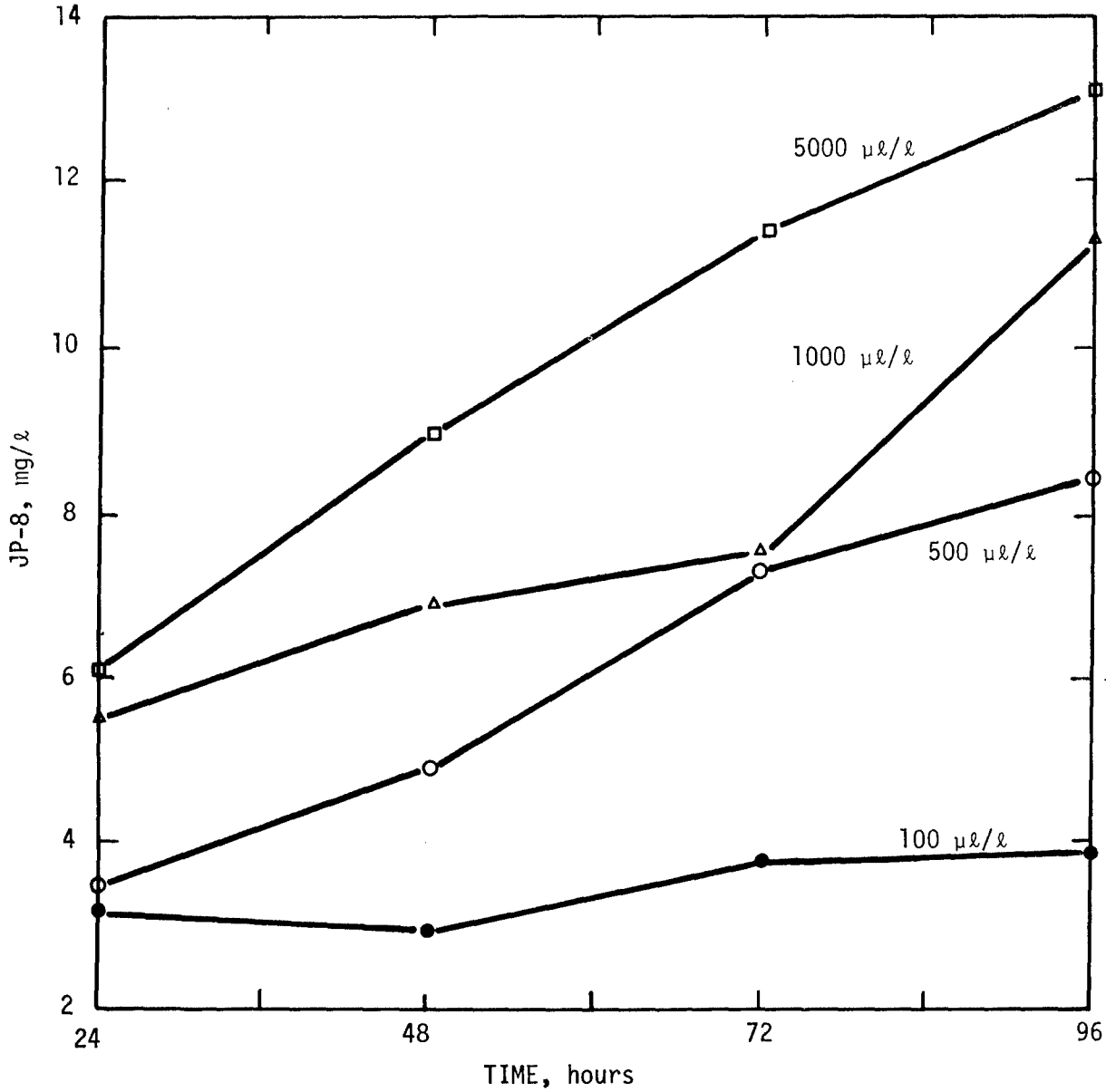


FIGURE 9. DISSOLUTION OF JP-8 AT A SERIES OF VOLUMETRIC ADDITIONS

Table 5

Survival of Golden Shiners in Static Bioassay with Floating Pools of Pure JP-8 Fuel in RFS Tap Water

JP-8 Added	Fish Survival, no.								
	Time, days								
µℓ/ℓ	0	1	2	3	4	5	6	7	8
0	10	10	10	10	9	9	9	9	9
10	10	10	10	10	10	10	10	10	10
50	10	10	10	10	10	10	10	10	10
100	10	10	10	10	10	10	10	9	9
500	10	10	9	7	3	1	1	1	0
1000	10	10	9	3	0	0	0	0	0
5000	10	10	8	2	0	0	0	0	0

concentration where mortalities have been observed in the previous studies. At the three highest concentrations of pure fuel, there was progressive dissolution of JP-8 throughout the 96-hr assay period. The 5000 µℓ/ℓ addition reached complete saturation of soluble JP-8 by 96 hours as shown by the JP-8 saturation data (see hardness studies).

Discussion

There was little indication from this study that physical encounters by fish with the floating JP-8 was a factor in mortality as previously noticed in studies of other fuels such as RJ-4 and RJ-5 (see 1976 Annual Report). Further work is necessary to draw firm conclusions on this point. The mean 96-hr LC50 of the soluble JP-8 fuel in hard, soft and tap water was 8.2 µℓ/ℓ. Based on the dissolution data, the LC50 for the pure fuel study at concentrations of 500 and 1000 µℓ/ℓ was on the order of 6-7 mg/ℓ. It may be quite fortuitous that these two sets of values are similar because the bioassay conditions in the two types of exposure are quite different. Thus in the fuel solution assay, the fish were exposed to a decreasing toxicant concentration throughout the assay period (due to volatility loss) while in the assay of pure fuel, the soluble fuel levels were constantly being replenished from a floating pool of pure fuel.

IV. JP-4 TOXICITY

Introduction

Since the previous work on acute toxicity of JP-4 to golden shiners the procedure for solubilization fuels has been modified by stirring the fuel-water mixture in a sealed container to insure no loss of volatile components. A direct comparison of JP-4 and JP-8 toxicities was made by conducting a JP-4 bioassay using the revised fuel solubilization procedure. The acute toxicity results of the previous study on water soluble JP-4 are also presented here for comparative purposes. Since GC techniques for quantifying fuel concentrations were not available at the time of this work, the results were reported as volumetric dilutions of a water saturated solution of JP-4. These volumetric dilutions may be converted to mg/ℓ JP-4 by assuming that equivalent solubilization was achieved as in the current procedure. Although there is a measure of uncertainty that equivalent solubilization was attained, the two solubilization processes were similar enough to warrant a reserved comparison.

Results of previous work with pure JP-4 can be compared with current pure JP-8 studies because the methodology has remained unchanged.

Results of Soluble JP-4 Bioassays

RFS tap water (total hardness = 28 mg $CaCO_3$ /ℓ), dechlorinated by aeration for 24 hours, was saturated with soluble JP-4 and diluted to volumetric percentages of 0, 12, 20, 32, 50 and 79%. Solutions were renewed daily during the 96-hr test period. Minimal aeration at rate of 70 bubbles per min was provided during the assay period.

From the fish survival data (Table 6), LC 50's were computed to be 40% (6.3 mg JP-4/ℓ) at 24 hours, 34% (5.3 mg JP-4/ℓ) at 48 hours, and 26% (4.1 mg JP-4/ℓ) at 96 hours. Confidence limits, computed by the Reed-Muench method, are presented in Figure 10. This computation gives LC50 values of 6.1, 5.2 and 3.8 mg/ℓ for 24, 48 and 96 hours, respectively.

The measured JP-4 concentration at each dilution is shown at 0 and 24 hours (Table 7) to indicate the volatility loss during a given 24-hr period. There is reasonably close agreement between computed and measured concentrations at the start of the experiment. The 24-hr concentrations indicate that there is a volatility loss of between 19-49% (mean = 35.4% ± 11.4%).

The previous study of the toxicity of water soluble components of JP-4 gave LC50 values of 31.3%, 26.7% , 21.2% and 20% for 24, 48, 72 and 96 hours, respectively. Assuming that the saturation concentration of soluble JP-4 was the same as in the current study (15.7 mg/ℓ), then these volumetric dilutions may be converted to mg/ℓ JP-4 to yield values of 4.9 mg/ℓ , 4.2 mg/ℓ, 3.3 mg/ℓ and 3.1 mg/ℓ for 24, 48, 72 and 96-hr LC50's, respectively. The comparison to the current work using Reed-Muench computations (Figure 11) shows that the results of the two studies

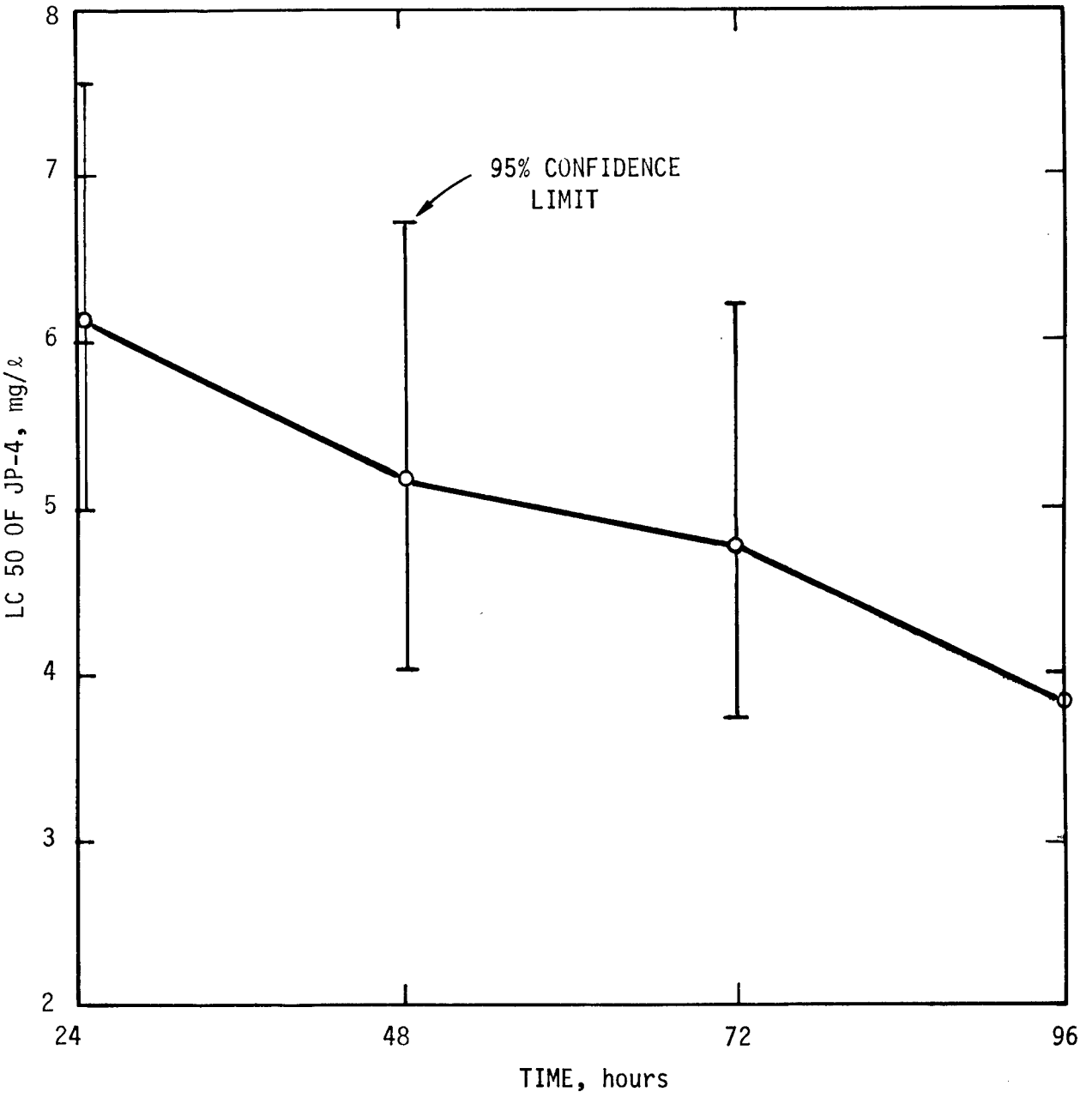


FIGURE 10. ACUTE TOXICITY OF SOLUBLE JP-4 COMPONENTS TO GOLDEN SHINERS (24-hr SOLUTION RENEWAL)

Table 6

Survival of Golden Shiners Exposed to Soluble JP-4

Soluble JP-4 Conc.		Fish Survival, No.			
Volume %	mg/ℓ	Time, hr.			
		24	48	72	96
0	0	10	10	10	10
12	1.9	9	9	9	8
20	3.1	10	10	10	10
32	5.0	9	6	5	1
50	7.9	1	0	0	0
79	12.4	0	0	0	0
100	15.7	-*	-	-	-

* No assay conducted at 100%

Table 7

Volatility of JP-4 During 24-hour Period

Volume %	Computed Concentration mg/ℓ	Measured Concentration mg/ℓ		Volatility Loss %
		0 hr.	24 hr.	
0	0	0	0	0
12	1.9	2.1	1.7	19
20	3.1	4.4	2.9	34
32	5.0	6.0	3.1	49
50	7.9	8.6	5.8	33
100	16.0	16.0	9.2	42

are in close agreement. The 95% confidence limit of the current study embraces the LC50 values of the previous study. There was little difference in fish size between the two sets of assays - in the previous assay, the fish averaged 4.3 cm in length and 1.20 g in wet weight, which is 5.5% smaller than the fish assayed in the current study.

Comparative Toxicity of Water Soluble JP-4 and JP-8

A comparison of JP-8 and JP-4 LC50 values computed by the Reed-Muench method for golden shiners (Table 8) indicates that JP-4 is much more acutely toxic than JP-8. The value of JP-4 LC50 from the current experiment is 3.8 mg/ℓ while the average 96-hr LC50 of JP-8 is 8.2 mg/ℓ.

Results of Pure Fuel Bioassays

A previous study on the exposure of golden shiners to JP-4 in the pure form gave values for toxicity in volumetric units which can be compared directly to the current work on pure JP-8 (Table 9).

These data demonstrate that JP-4 was more toxic at 24, 48 and 72 hours, but after 96 hours there was no significant difference in toxicity.

The rate of JP-4 solubilization into water from surface pools of floating JP-4 was investigated using the same procedure as that employed in JP-8 solubilization. Four assay vessels containing 15 ℓ of 100, 500, 1000 and 5000 μℓ/ℓ pure JP-4 fuel in RFS tap water respectively were set up with no fish in them. The vessels were aerated gently at 70 bubbles per minute for 96 hours to simulate the bioassay procedure.

The results presented indicate that JP-4 dissolves rapidly (Figure 12) and appears to reach saturation after only 24 hours in the 100, 500 and 1000 μℓ/ℓ JP-4 mixtures. In these three mixtures there appears to be insufficient fuel in the surface reservoir to reach and maintain the saturation concentration of 15.7 mg/ℓ . The 5000 μℓ/ℓ mixture contains sufficient fuel to approach this saturation concentration and by 96 hours was 14.1 mg/ℓ .

Discussion

Static acute toxicity studies of water saturated with the soluble components of JP-4 and JP-8 indicated that JP-4 with a 96-hr LC50 of 3.8 mg/ℓ was about twice as toxic as JP-8 (96-hr LC50, 8.2 mg/ℓ). Pronounced stress symptoms appeared in golden shiners exposed to sublethal concentrations of both fuels. These symptoms were most pronounced on the initial exposure of the fish to fuel solutions and tended to decrease during the 24-hr period of exposure following solution renewal. These observations were indicative of volatility loss and GC analyses confirmed that volatility losses were indeed significant during a 24-hr period.

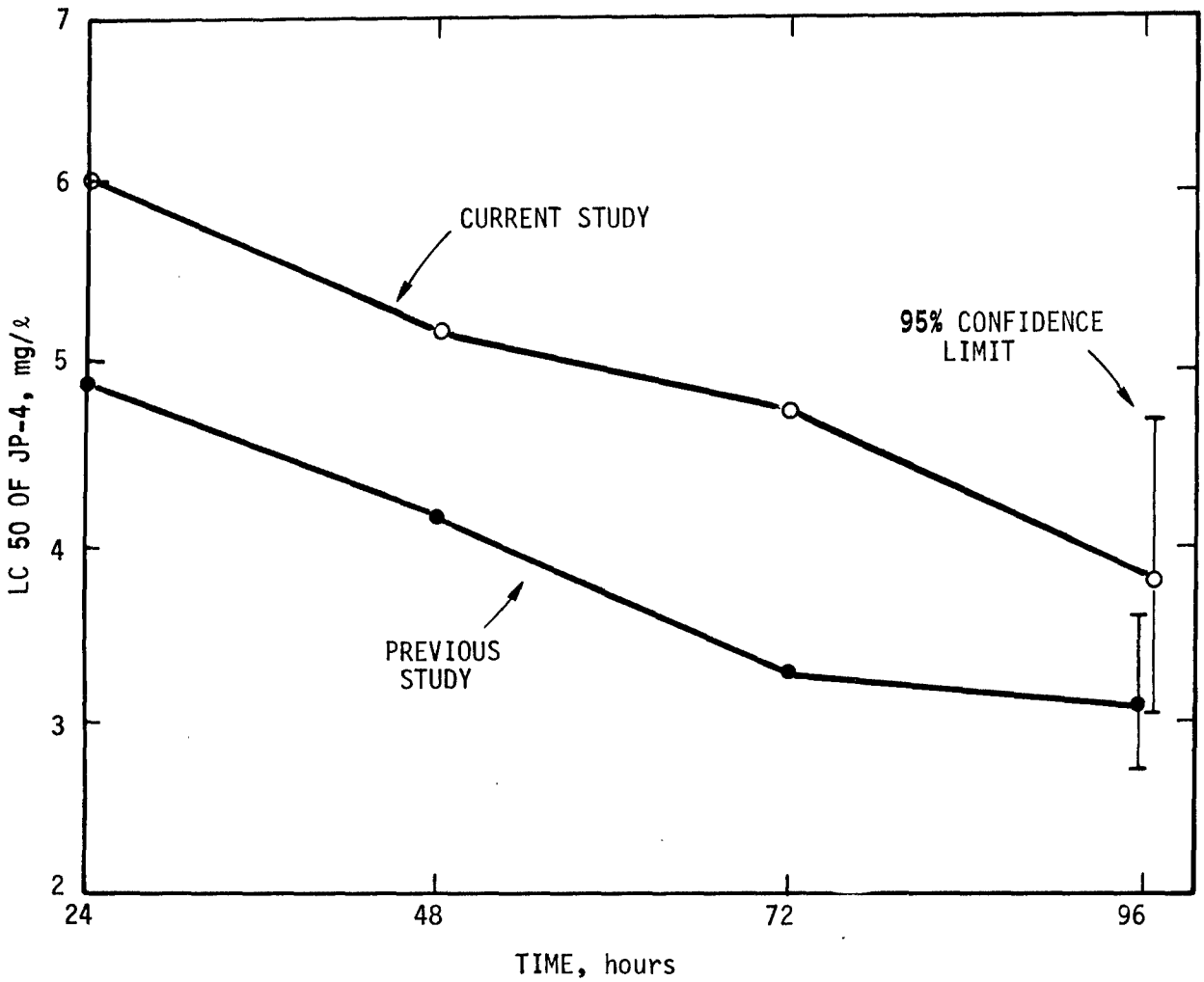


FIGURE 11. COMPARISON OF SOLUBLE JP-4 ACUTE TOXICITY STUDIES
(TEST ORGANISM: GOLDEN SHINER)

Table 8

Comparison of Acute Toxicity of Water Soluble Components of JP-8 and JP-4 to Golden Shiners

Study	96-hour LC50 mg/ℓ	95% Confidence Limits
JP-8 (soft water)	8.4	6.8 - 10.4
JP-8 (tap water)	8.5	7.7 - 9.5
JP-8 (hard water)	7.7	6.7 - 8.9
Average	8.2	7.1 - 9.6
JP-4 (current)	3.8	3.1 - 4.7

Table 9

Comparison of Acute Toxicity of Pure JP-4 and JP-8 to Golden Shiners

Fuel	LC50, μℓ/ℓ Time, hr.				95% C.L. 96-hour LC50 μℓ/ℓ	Mean Fish Weight g	Average Standard Length cm
	24	48	72	96			
JP-4	1600	620	570	570	400 - 800	0.87	4.17 ± 0.22
JP-8	-*	-*	780	316	163 - 610	1.27	4.55 ± 0.08

* No LC50 (50% deaths not evident at any fuel concentration)

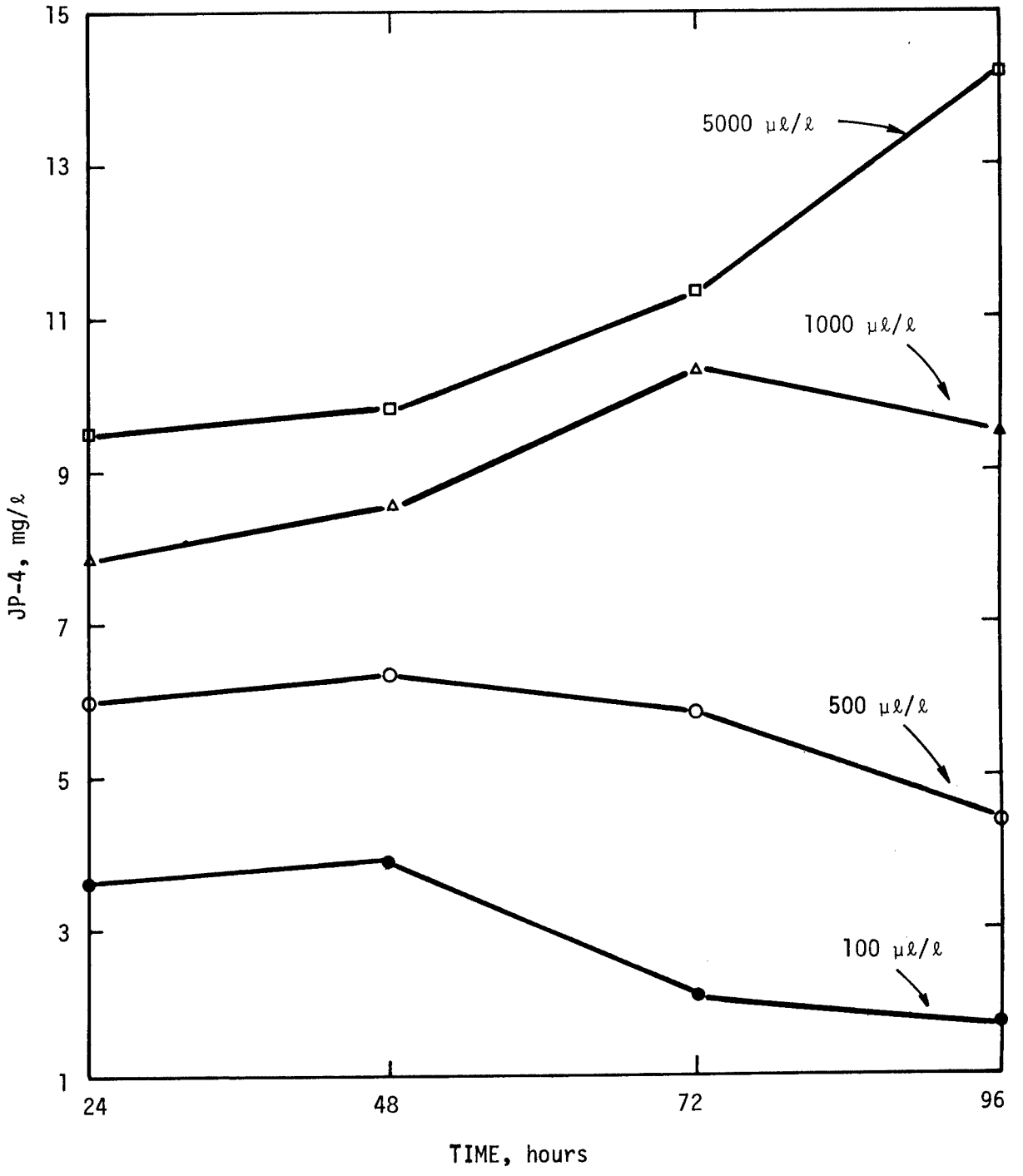


FIGURE 12. SOLUBILIZATION OF A SERIES OF ADDITIONS OF PURE FUEL JP-4 WITH TIME

Because of this there is a need to conduct continuous acute toxicity studies in which volatility losses can be controlled. Apparatus for this purpose has been constructed and preliminary studies are currently underway.

In the pure fuel form, the acute toxicity of JP-4 was significantly greater than JP-8 for the first 48 hours, but by 96 hours, there was no significant difference in the toxicities. These results can be explained by the different rates at which the two fuels dissolve in water. JP-4 initially dissolves much more rapidly than JP-8, but by 96 hours, the soluble JP-8 concentration is higher (8 mg/ℓ) than the soluble JP-4 concentration (4.5 mg/ℓ).

Fish stressed at sublethal fuel concentrations, appear to recover fully from these symptoms when full concentration diminishes. Since fish are prone to swim to non-polluted water, they likely would swim away from a fuel spill if the opportunity to escape was available to them. Thus escape and avoidance could result in recovery in a natural situation.

In a mixture of 5000μℓ/ JP-8/ℓ of water, the rate of fuel dissolution measured in these experiments would produce a soluble JP-8 concentration of 6 mg/ℓ after the first day and an additional 2 mg/ℓ/day for the following three days. Since no fish mortality occurred until after the first day, there would perhaps be time for fish to swim away from a spill even if the spill occurred in relatively quiescent waters.

The fact that volatilization does not preferentially remove toxic components indicates that volatility would not serve to materially enhance the survival success of fish in an aquatic region where a floating pool of fuel is present. In areas remote to the spill, volatility loss of soluble fuel would be expected to be helpful since the overall concentration would decrease with time and even moribund fish might be able to recover as the concentration of soluble JP-8 decreases by volatility loss.

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